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(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Preventing Allograft Rejection with Antibodies to Adhesion Molecules

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(54) Title: PREVENTING ALLOGRAFT REJECTION WITH ANTIBODIES TO ADHESION MOLECULES

(57) Abstract

Compositions and methods for prolonging the function of a transplanted allograft and preventing transplant rejection are provided. Particularly, tolerance is induced in a recipient mammal to a transplanted organ or tissue by treatment with a composition comprising more than one adhesion molecule inhibitor including antibodies to the adhesion molecule and corresponding ligand, e.g. antibodies to LFA-1 and ICAM-1. Compositions of the invention find additional use in treating inflammatory reactions, as well as allergies and autoimmune diseases.

WHAT IS CLAIMED IS:

1. A method for preventing allograft rejection or prolonging the function of a transplanted allograft, said method comprising administering to a mammal a therapeutically effective amount of a composition comprising at least two anti-adhesion molecule antibody inhibitors, wherein at least one inhibitor is specific to the receptor of a receptor-ligand pair and at least one inhibitor is specific to the ligand of the receptor-ligand pair.

2. A method according to Claim 1, wherein said anti-adhesion molecule antibody inhibitors are selected from antibodies to LFA-1, ICAM-1, Mac-1, CR3, CR4, LeuM5, VCAM-1, VLA-4, ELAM-1, CD-2 and LFA-3.

3. A method according to Claim 2, wherein said antibody is a monoclonal antibody.

4. A method according to Claim 1, wherein the inhibitor specific to the receptor of the receptor-ligand pair is an antibody directed against LFA-1 and the inhibitor specific to the ligand of the receptor-ligand pair is an antibody directed against ICAM-1.

5. A method according to Claim 1, wherein the inhibitor specific to the receptor of the receptor-ligand pair is an antibody directed against VLA-4 and the inhibitor specific to the ligand of the receptor-ligand pair is an antibody directed against VCAM-1.

6. A pharmaceutical composition for use in therapy comprising at least two anti-adhesion molecule antibody inhibitors, wherein at least one inhibitor is specific to the receptor

of a receptor-ligand pair and at least one inhibitor is specific to the ligand of the receptor-ligand pair.

7. A composition according to Claim 6, wherein said anti-adhesion molecule antibody inhibitors are selected from antibodies to LFA-1, ICAM-1, Mac-1, CR3, CR4, LeuM5, VCAM-1, VLA-4, ELAM-1, CD-2 and LFA-3.

8. A composition according to Claim 7, wherein said antibody is a monoclonal antibody.

9. A composition according to Claim 6, wherein the inhibitor specific to the receptor of the receptor-ligand pair is an antibody directed against LFA-1 and the inhibitor specific to the ligand of the receptor-ligand pair is an antibody directed against ICAM-1.

10. A composition according to Claim 6, wherein the inhibitor specific to the receptor of the receptor-ligand pair is an antibody directed against VLA-4 and the inhibitor specific to the ligand of the receptor-ligand pair is an antibody directed against VCAM-1.

11. A composition according to any one of Claims 6 to 10 for use in preventing allograft rejection or prolonging the function of a transplanted allograft.

12. Use of the anti-adhesion molecule antibody inhibitors as defined in any one of Claims 6 to 10 for the manufacture of a medicament for use in preventing allograft rejection or prolonging the function of a transplanted allograft.

Preventing allograft rejection with antibodies to adhesion molecules.

Field of the Invention

This invention relates to the field of transplantation, particularly to methods for preventing allograft rejection.

Background of the Invention

Transplantation of organs and tissues is an important aspect of treating end-stage organ failure and replacing damaged tissue. The use of allogeneic, or non-self, transplantation tissue has become increasingly important in medicine. The use of allografts, however, is limited by the frequent rejection of the graft tissue by the recipient host, because of antigenic differences between the donor and the recipient.

The antigenic differences between individual members of the same species are referred to as "alloantigens." When alloantigens are involved in rejection of allogeneic tissue grafts, they are referred to as "histocompatibility antigens." The terms "major histocompatibility antigens" and "major histocompatibility complex" (MHC) refer to the products of a single closely-linked region of genes.

Graft rejection is the consequence of the host immune response to histocompatibility antigens expressed by the graft tissue. Allografts generally survive for a period of days to weeks, but may subsequently become inflamed and infiltrated with lymphocytes and monocytes. The graft tissue eventually becomes necrotic, and in the case of skin transplants, is sloughed from the skin. However, in the case of a vital organ such as the heart, the sequelae to tissue rejection can be fatal to the recipient.

Cyclosporine is a cyclic, nonwater-soluble, highly nonpolar molecule composed of 11 amino acids. Cyclosporine is widely used for prolonging the function of various

transplanted organs. Its immunosuppressive effects selectively inhibit T-cell function, allowing survival of allografts without myelosuppression, i.e., heart transplants (see Meyers et al., N. Engl. J. Med. 311:699 (1984)).

One of the major disadvantages in conventional immunosuppressants including cyclosporine and steroid is the generalized suppression of host immunity, which causes serious opportunistic infection in patients. To overcome the serious side effects of conventional drugs, antigen- specific immunosuppression is strongly desired.

Also, the use of cyclosporine is somewhat limited, both by its association with infection and also because of hepatic and renal toxicities. Clinical use of cyclosporine is associated with reversible, dose-related increases in blood urea nitrogen (BUN) and serum creatinine levels and depression of creatinine clearance. Some nephrotoxicity is reported to occur in almost 80% of renal transplant patients using cyclosporine.

The irreversible cyclosporine-induced deterioration of renal function has been described in heart transplant patients (Meyers et al., N. Engl. J. Med. 311:699 (1984)). Possible irreversible histological findings in kidneys of transplant patients given cyclosporine therapy have also been published (Mihatsch et al., Transplant Proc. 15:2821 (1983)).

Thus, deterioration of renal function is a major side effect which reduces the practical clinical therapeutical efficacy of cyclosporine treatment for transplant and non-transplant patients. Thus, a need exists for an improved method for inducing graft tolerance in mammalian recipients, particularly humans.

Related Art

A review article on the adhesion receptors of the immune system is provided by Springer, T.A., Nature 346:425 (1990).

Springer et al., Ann. Rev. Immunol. 5:223-252 (1987), discuss cell adhesion receptors, LFA-1, CD2, and LFA-3 molecules, of the immune system. In particular, prevention of graft rejection utilizing monoclonal antibodies to LFA-1 is discussed.

Dijken et al., Transplantation 49:882-886 (1990), describe the *in vivo* use of a monoclonal antibody to LFA-1 to prevent rejection of T cell depleted allogenic bone marrow.

Benjamin discusses mechanisms of monoclonal antibody-facilitated tolerance induction. In particular, mice given a short parenteral course of a monoclonal antibody to the CD4 molecule on T helper cells became tolerant to certain protein antigens administered simultaneously.

Vang and Rock, J. Immunol. 146:3273-3279 (1991), report that engagement of the sIg receptor induces the expression and function of both ICAM-1 and LFA-1 on B lymphocytes.

Discussion of lymphocyte function-associated antigen 1 (LFA-1) can be found in Marlin and Springer, Cell 51:813-819 (1987), and Davignon et al., Proc. Natl. Acad. Sci USA 78:4535-4539 (1981).

SUMMARY OF THE INVENTION

Compositions and methods for prolonging the function of a transplanted allograft and preventing transplant rejection are provided. The method comprises administering a composition comprising more than one inhibitor of adhesion molecule inhibitor. Preferably, inhibitors of an adhesion molecule and its counter-receptor molecule are utilized. The inhibitors include antibodies to adhesion molecules and receptor ligands.

Compositions additionally find use in inflammatory reactions as well as allergies and autoimmune diseases.

DETAILED DESCRIPTION OF THE INVENTION

The invention is drawn to compositions and methods for prolonging graft survival in a host. Thus, the invention provides a means for preventing allograft rejection.

The compositions comprise adhesion molecule inhibitors. By "adhesion molecule inhibitor" is intended a molecule which inhibits the activation of T cells and/or B cells. Such inhibitors act to prevent intercellular adhesion in immunological and inflammatory reactions. Such inhibitors include, for the most part, antibodies to adhesion molecules or their receptor ligands. A variety of cellular adhesion and recognition molecules are known in the art. These include, but are not limited to, the leukocyte integrins, for example, LFA-1 (Lymphocyte function- associated antigen-1), MAC-1 (macrophage antigen-1), VLA-4 (very late antigen-4), CR3 (complement receptor type-3), CR4 (complement receptor type-4), LeuM5, and the like. See, for example, Kishimoto et al., Adv. Immunol. 46:149-182 (1989); Nishirnura et al., Cell Immunol. 107:32-39 (1987); Benjamin et al., Eur. J. Immunol. 18:1079-1088 (1988); Davignon et al., Proc. Natl. Acad. Sci. USA 78:4535-4539 (1981); Marlin and Springer, Cell 51:813-819 (1987); Springer et al., Ann. Rev. Immunol. 5:223-252 (1987); Dijken et al., Transplantation 49:882-886 (1990); Diamond et al., Cell 65:961-971 (1991); and the references cited therein. The cellular adhesion molecules include, for the most part, cell surface glycoproteins that promote intercellular adhesion in immunological and inflammatory reactions. Other adhesion or receptor molecules include, for example, LFA-3, ICAM-1, ICAM-2, VCAM-1, ELAM-2, CD-2, p150,95, and others.

The compositions of the invention comprise more than one inhibitor molecule to the adhesion molecules or receptors. It is preferred that the composition include inhibitors to the receptor and the ligand of a receptor- ligand pair (for example, an inhibitor of LFA-1 utilized with an inhibitor of ICAM-1, and an inhibitor of VLA-4 utilized with an inhibitor of VCAM-1).

Inhibitors of the invention prevent the adhesion molecules from creating the immune response. T cell immune recognition requires adhesion receptors as well as the T cell receptor by promoting attachment of T cells to their targets and transduce regulatory signals to the T cell. Inhibitor molecules prevent the activation of antigen receptors on the T cell or B cell. Preferred inhibitors include antibodies to adhesion receptors or ligands. The term "antibodies" includes both polyclonal and monoclonal intact molecules as well as fragments thereof, such as, for example, Fab, $F(ab)_2$, Fv, which are capable of binding antigen.

Particular antibodies are known in the art for adhesion molecules and receptor ligands. These include those provided by Benjamin et al., Eur. J. Immunol. 18:1079-1088 (1988); Spring et al., Ann. Rev. Immunol. 5:223-52 (1987); and Dijken et al., Transplantation 49:882-886 (1990).

However, once adhesion or receptor molecules have been identified, methods are available in the art for the production of antibodies which bind to the adhesion molecules. Several methods are available in the art for producing antibodies or antibody fragments. It is recognized that any such method could be utilized to make the antibodies of the present invention. See, for example, Kohler and Milstein, Nature 256:496 (1975); Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1988); Davis et al., Biotechnology 9:165-169 (1991); Buchner and Rudolph, Biotechnology 9:157-162 (1991);

and the references cited by these articles. Standard reference works setting for the general principles of immunology include the work of Klein, J., Immunology: The Science of Cell-Non-cell Discrimination, John Wiley & Sons, New York (1982); Kenneth et al., Monoclonal Antibodies, Hybridoma: A New Dimension in Biological Analyses, Plenum Press, New York (1980); CampbelL A., "Monoclonal Antibody Technology," In: Laboratory Techniques in Biochemistry and Molecular Biology, 13, Burdon et al. (eds.), Alsevier, Amsterdam (1984); and Eisen, H.N., In: Microbiology, 3rd Ed., Davis et al. (eds.), Harper & Row, Philadelphia (1980).

As noted, both polyclonal and monoclonal antibodies may be employed in accordance with the present invention. Furthermore, antibodies or their functional derivatives, which are produced in humans or are humanized (i.e., not immunogenic in a human) by recombinant or other technology, may be utilized. Humanized antibodies may be produced, for example, by replacing an immunogenic portion of an antibody with a corresponding, but not immunogenic, portion (i.e., chimeric antibodies). See, Robinson et al., International Patent Publication PCI/US86/02269; Akira et al., European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al., European Patent Application 173,494; Neuberger, PCI Application WO86/01533; Kabilly et al., European Patent Application 125,023; Better et al., Science 240:1041-1043 (1988); Sun et al., Proc. Natl. Acad. Sci. USA 84:214-218 (1987); Mishirnura et al., Cancer Res. 47:999-1005 (1987); Wood et al., Nature 314: 446-449 (1985); and Shaw et al., J. Natl. Cancer Institute 80:1553-1559 (1988). For general reviews of humanized chimeric antibodies, see, Morrison, S.L., Science 229: 1202-1209 (1985), and Oi et al., Biotechniques 4:214 (1986).

As noted, the inhibitors are utilized before or after allograft transplantation to prevent rejection. The methods

of the invention can be utilized with any allograft, either organ or tissue, including but not limited to, heart, kidney, liver, bone marrow cells, skin, and the like.

The inhibitor compositions of the invention can be administered parenterally by injection, rapid infusion, nasopharyngeal absorption (intranasopharyngeally), derma absorption, or orally. The compositions may alternatively be administered intramuscularly or intravenously. Compositions for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of nonaqueous solvent are poly-propylene, glycol, polyethylene, glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyloleate. Carriers or occlusive dressings can be used to increase skin permeability and enhance absorption.

The inhibitor compositions may be utilized alone or in combination with other therapeutic agents. The compositions of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions such as by admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation are described, for example, in Remington's Pharmaceutical Sciences (16th Ed.), Osol, A., ed., Mack, Easton, PA (1980). In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of an inhibitor, either alone or in combination, with a suitable carrier vehicle.

Additional pharmaceutical methods may be employed to control the duration of action. Controlled release preparations may be achieved by the use of polymers to complex or absorb the antibody or antibody fragments/ therapeutic compositions of the present invention.

It is contemplated that the therapeutic or diagnostic compositions of the present invention will be administered to

an individual in therapeutically effective amounts. That is, in an amount sufficient to prolong the function of a transplanted allograft and prevent transplant rejection. The effective amount of the composition will vary according to the weight, sex, age, and medical history of the individual. Other factors which influence the effective amount may include, but are not limited to, the severity of the patient's condition, the type of allograft, the kinetics of interactions between the target protein and the therapeutic composition, etc. Generally, the composition will be administered in doses ranging from about 1 μg to about 200 mg antibodies, more generally about 50 μg to about 100 mg. Animal models may be utilized to further define specific dosages.

The antibody/inhibitor molecules of the present invention may be dissolved in any physiologically tolerated liquid in order to prepare an injectable bolus. It is preferable to prepare such a bolus by dissolving the molecule in normal saline.

The pharmaceutical compositions of the invention are generally utilized to treat a transplant recipient before and/or following transplantation. The treatment may be repeated to maintain the function of a transplanted allograft. Generally, the composition is administered before transplantation or immediately following the transplant operation. The duration of treatment may vary from about several hours to several weeks depending upon the patient's condition. Alternatively a series of treatments may be given for the first hours, days or weeks following transplant. Once the initial treatment or series of treatments is completed, the composition will only be administered occasionally. That is, after the initial treatments, the composition will only be administered upon the development of complications or the indication of transplant rejection.

As adhesion molecules are involved in the inflammatory response, it is recognized that the methods of the present invention can be utilized to treat inflammatory reactions. The method can further be utilized to suppress autoimmune diseases, or other T-cell mediated responses.

Having now generally described this invention, the same will be better understood by reference to certain specific examples which are included herein for purposes of illustration only, and are not intended to be limiting of the invention, unless specified.

EXPERIMENTAL

Recent advances in the investigation on adhesion molecules reveal critical roles of cell adhesions in creating immune response (Springer et al., Ann. Rev. Immunol. 5:223-252 (1987); Springer, T.A., Nature 364:425-433 (1990)). T cell immune recognition requires adhesion receptors as well as the T cell receptor by promoting attachment of T cells to their targets and transduce regulatory signals to the T cell. Lymphocyte function associated antigen 1 (LFA-1) and intercellular adhesion molecule 1 (ICAM-1) form one such critical heterophilic adhesive receptor-ligand pair (Marlin and Springer, Cell 51:813-819 (1987)). LFA-1 is required for a broad range of leukocyte functions, including T cell proliferation (Davignon et al., Proc. Natl. Acad. Sci. USA 78:4535-4539 (1981)) and T-helper and B lymphocyte responses (DeFranco, A.L., Nature 351:603-604 (1991)). Activation of antigen receptors on the T cell (Springer, T.A., Nature 364:425-433 (1990)) or B cell (Dang and Rock, J. Immunol. 146(10):3273-9 (1991)) causes LFA-1 to bind its ligand with higher affinity. Also, interaction of LFA-1 and ICAM-1 is required for optimal T cell function in vitro (Makgoba et al., Eur. J. Immunol. 18:637-640 (1988); Dustin and Springer, Nature 341:619-624 (1989)). Therefore, monoclonal antibodies

directed against these antigens are potential agents for the prevention of graft rejection (Benjamin et al., Eur. J. Immunol. 18:1079-1088 (1988); Cosimi et al., J. Immunol. 144:4604-4612 (1990); van Dijken et al., Transplantation 49:882-886 (1990)). In this report, we demonstrate for the first time the strong effects of these antibodies on allograft survival using a mouse heterotopic cardiac allograft model.

The monoclonal antibodies used in this study, KBA (IgG2a) (Nishimura et al., Cell. Immunol. 107(1):32-9 (1987); Nishimura et al., Cell. Immunol. 94:122-132 (1985)), M18/2 (IgG2a) (Sanchez et al., J. Exp. Med. 158(2):586-602 (1983)), and YN1/1.7 (IgG2b) (Takei, F., J. Immunol. 134: 1403-1407 (1985); Prieto et al., Eur. J. Immunol. 19(9): 1551-7 (1989)) are rat immunoglobulin directed against mouse CD11a (a chain of LFA-1), CD18 (β chain of LFA-1) and ICAM-1, respectively. Hybridoma cells which produce these antibodies were cultured in RPMI1640 supplemented with 10% fetal bovine serum and 0.1% gentamicin. Monoclonal antibodies were purified using Protein G affinity column from ascites of nude mice that were injected with these hybridomas.

Balb/c ($H2^d$) (All animals were purchased from Charles River Resources (Boston). All animal experiments were approved by the Committee on Research Animal Care Protocol Review Group and carried out according to Massachusetts General Hospital guidelines.) Hearts were heterotopically transplanted into C3H/He ($H21^k$) recipients by a microsurgery technique (Isobe et al., Circulation (1991, in press)). Survival of cardiac graft was assessed by daily palpation and the cessation of graft beat was interpreted as the completion of rejection (Isobe et al., Circulation (1991, in press)). Treatment was performed by daily intraperitoneal injection of purified antibodies starting right after operation for six days.

Because of the full incompatibility of H2 complex, control mice without any immunosuppression invariably rejected allografts within ten days (Table 1). Animals treated with the daily doses of 100 μg of either YN1/1.7 or KBA showed significant prolongation of allograft survival as evidenced by persistence of graft beat, when compared with control mice; however, all these animals subsequently rejected allografts within 50 days. Animals treated with same amount of M18/2 did not show any increase in graft survival. In contrast to the results observed with either YN1/1.7 or KBA alone, all six animals treated with 50 μg of YN1/1.7 together with 50 μg of KBA accepted cardiac allografts as long as the observation was continued (75 to 150 days). The intensity and frequency of beating of these allografts were the same as that of isografts.

Histological analysis performed on C3H/He recipients of Balb/c heart allografts showed greatly reduced mononuclear cell infiltration of grafts treated with the two antibodies as compared with untreated controls. Seven days after transplantation with no immunosuppressive treatment, a control allograft showed massive infiltration of leukocytes together with myocyte necrosis and interstitial hemorrhage. This result is in sharp contrast to allograft recipients treated with a six-day course of YN1/1.7 and KBA monoclonal antibody starting right after transplantation. At seven days post-transplantation, these animals showed diffuse interstitial leukocyte infiltration, (grade IA rejection (Billingham et al., J. Heart Transplant 9(6):587-593 (1990)), and the myocytes were free of necrosis. Allografts examined 40, 75 and 120 days after transplantation showed only scattered areas of fibrosis and showed no evidence of active rejection.

Cell mediated cytotoxic activity of recipients' splenocytes was tested at the seventh, 40th and 75th day of transplantation (Table 2). At the seventh day, spleen cells

from allografted recipient mice without immunosuppressive treatment revealed cytotoxic activity against tumor cells that bear donor syngeneic MHC antigens. Allografted mice treated with KBA or treated with both KBA and YN1/1.7 did not show any increase in cytotoxic activity when compared with that of normal virgin mice. Mice treated with YN1/1.7 showed intermediate results. These observations for KBA/YN1/1.7 treated mice were consistent at 40 and 75 days.

To further evaluate the tolerant state of these mice, they were challenged with skin grafting. Four mice with long survived cardiac allografts (65 to 72 days) were transplanted with donor syngeneic (Balb/c) and third party (C57BL/6, $H2^b$) body skin simultaneously. All animals normally rejected third party skin between 11 and 14 days after transplantation; however, they accepted donor syngeneic skin more than 60 days, or as long as observation was made. All cardiac grafts kept beating during observation. The results clearly indicate antigenspecific tolerance was present in these mice.

Indirect immunofluorescence staining to investigate LFA-1 and ICAM-1 expression on splenocytes of allografted mice demonstrated that the mAb treatment led to down-modulation of the respective antigens on the cell surface at day 7 post transplantation. This down regulation accounts for the inability to detect alloreactive cytotoxic T lymphocyte activity at day 7, and could also account for the induction of tolerance against alloantigens. The expression of LFA-1 and ICAM-1 returned to normal levels 40 days after transplantation, while alloreactive cytotoxic T lymphocyte activity was still undetectable.

The mechanism of this sustained unresponsiveness is to be established. As cell adhesion by LFA-1/ICAM-1 is an essential part of T cell function (Springer et al., Ann. Rev. Immunol. 5:223-252 (1987); Springer, T.A., Nature 364: 425-433 (1990)), it is reasonable to speculate that the adhesion mediated by

LFA-1 and ICAM-1 plays a crucial role in the initiation of immune response against alloantigens. Temporal blocking of this adhesion system together with massive introduction of alloantigen is likely to facilitate the induction of specific unresponsiveness. The evidence that the population of CD11a positive cells returned to normal range at the chronic stage implies that the tolerance is maintained by some mechanisms other than elimination of LFA-1 and ICAM-1 molecules.

A most interesting finding in this experiment is that anti-ICAM-1 and anti-CD11a antibodies appear to work synergistically to induce tolerance. It has already been shown that each antibody used in this experiment completely blocks in vitro cell mediated cytotoxicity (Nishimura et al., Cell Immunol. 107(1):32-9 (1987); Nishimura et al., Cell Immunol. 94:122-132 (1985); Prieto et al., Eur. J. Immunol. 19(9):1551-7 (1989)). However, our in vivo experiments showed these antibodies have only a modest effect on graft survival prolongation when they are injected individually. Complete acceptance of graft was achieved only after simultaneous administration of the two antibodies. Although this synergism is a matter of further investigation, the fact that LFA-1 has at least three ligands, ICAM-1, ICAM-2 (Staunton et al., Nature 339:61-64 (1989)), and an unknown third one (de Fougerolles et al., J. Exp. Med. 174:253-267 (1991)) may partly explain this synergism. Also, ICAM-1 has another counter-receptor, Mac-1 (Diamond et al., Cell 65:961-971 (1991)), which is expressed primarily on myeloid and natural killer cells (Kishimoto et al., Adv. Immunol. 46:146-182 (1989)). Although the roles of these adhesion molecules in rejection have not been determined, because of this complexity, interference of cell adhesion is most effective after blocking of both sides of an adhesion pair.

Whatever the mechanism is, these observations clearly indicate the importance of the ICAM-1/LFA-1 adhesion in the

pathogenesis of rejection and suggest a rationale for application of this mode of immunosuppression in patients.

TABLE 1. Survival days of cardiac allografts (Balb/c) transplanted into C3H/He mice. Heterotopic cardiac transplantation was made by a microsurgical technique (Cosirni et al., J. Immunol. 144:4604-4612 (1990)). Recipient mice were injected daily with either 100 μg of YN1/1.7, 100 μg of KBA, or 50 μg of YN1/1.7 plus 50 μg of KBA starting right after operation until 5th day of transplantation. Survival time of YN1/1.7 and KBA treated mouse was significantly ($p<0.05$) greater than that of either control (no immunosuppression), YN1/1.7 or KBA treated mice.

treatment	n	survival days	mean survival time ± SD
none	6	7,7,8,8,8,10	8.0 ±1.1
YN1/1.7	6	11,12,12,13,15,23	14.3 ±4.5
KBA	5	17,20,25,38,47	29.4 ±12.7
M18/2	6	7,8,9,9,10,10	8.8 ±1.2
YN1/1.7 plus KBA	6	>70,>70,>70,>70, >70,>70	>70

TABLE 2. Cytotoxic T lymphocyte assay. Recipient C3H/He mice were sacrificed at 7, 40 or 75 days after transplantation of Balb/c mouse heart. They received 100 μg of either YN1/1.7, KBA, or 50 μg each of the two antibodies daily starting the day of transplantation until the 5th day. Fresh spleen cells were washed three times after a lysis of red blood cell by 175 Mm ammonium chloride. Standard 4h cell mediated lympholysis assay was performed using P815 cells labeled with ^{51}chromium as target cells ($4x10^4$/well). Results are expressed as percent lysis. Data are average of triplicate and spontaneous release was 15-25% of maximal release in all experiments. The experiment was repeated with consistent results.

treatment	cardiac transplant	days after operation	effector/target 5	effector/target 20
none	+	7	63	20.8
YN1/1.7	+	7	5.2	15.4
KBA	+	7	0.1	6.3
YN1/1.7 plus KBA	+	7	1.2	83
YN1/1.7 plus KBA	+	7	2.5	8.3
YN1/1.7 plus KBA	+	40	1.8	3.6
none	-	75*	2.3	6.5

*The recipient mouse was transplanted with donor syngeneic and third party skin 8 days before the cytotoxic assay.

Modifications of the above-described modes for carrying out the invention that are obvious to persons of skill in medicine, immunology, hybridoma technology, pharmacology, and/or related fields are intended to be within the scope of the following claims.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

SUBSTITUTE

REMPLACEMENT

SECTION is not Present

Cette Section est Absente